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Buss, A

Abstract: Axons undergo Wallerian degeneration (WD) distal to a point of injury. In the lesioned PNS, WD may be followed by successful axonal regeneration and functional recovery. However, in the lesioned mammalian CNS, there is no significant axonal regeneration. Myelin-associated proteins (MAPs) have been shown to play significant roles in preventing axonal regeneration in the CNS. Since relatively little is known about such events in human CNS pathologies, we performed an immunohistochemical investigation on the temporal changes of four MAPs during WD in post-mortem spinal cords of 22 patients who died 2 days to 30 years after either cerebral infarction or traumatic spinal cord injury. In contrast to experimental studies in rats, the loss of myelin sheaths is greatly delayed in humans and continues slowly over a number of years. However, in agreement with animal data, a sequential loss of myelin proteins was found which was dependent on their location within the myelin sheath. Myelin proteins situated on the peri-axonal membrane were the first to be lost, the time course correlating with the loss of axonal markers. Proteins located within compact myelin or on the outer myelin membrane were still detectable 3 years after injury in degenerating fibre tracts, long after the disappearance of the corresponding axons. The persistence of axon growth-inhibitory proteins such as NOGO-A in degenerating nerve fibre tracts may contribute to the maintenance of an environment that is hostile to axon regeneration, long after the initial injury. The present data highlight the importance of correlating the well documented, lesion-induced changes that take place in controlled laboratory investigations with those that take place in the clinical domain

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Sequential loss of myelin proteins during Wallerian degeneration in the human spinal cord

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Summary

Axons undergo Wallerian degeneration (WD) distal to a point of injury. In the lesioned PNS, WD may be followed by successful axonal regeneration and functional recovery. However, in the lesioned mammalian CNS, there is no significant axonal regeneration. Myelin-associated proteins (MAPs) have been shown to play significant roles in preventing axonal regeneration in the CNS. Since relatively little is known about such events in human CNS pathologies, we performed an immunohistochemical investigation on the temporal changes of four MAPs during WD in post-mortem spinal cords of 22 patients who died 2 days to 30 years after either cerebral infarction or traumatic spinal cord injury. In contrast to experimental studies in rats, the loss of myelin sheaths is greatly delayed in humans and continues slowly over a number of years. However, in agreement with animal data, a sequential loss of myelin proteins was found which was dependent on their location within the myelin sheath. Myelin proteins situated on the peri-axonal membrane were the first to be lost, the time course correlating with the loss of axonal markers. Proteins located within compact myelin or on the outer myelin membrane were still detectable 3 years after injury in degenerating fibre tracts, long after the disappearance of the corresponding axons. The persistence of axon growth-inhibitory proteins such as NOGO-A in degenerating nerve fibre tracts may contribute to the maintenance of an environment that is hostile to axon regeneration, long after the initial injury. The present data highlight the importance of correlating the well documented, lesion-induced changes that take place in controlled laboratory investigations with those that take place in the clinical domain.

Keywords: Wallerian degeneration; spinal cord; oligodendrocyte; myelin; NOGO-A

Abbreviations: CST = corticospinal tract; MAG = myelin-associated glycoprotein; MBP = myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; PLP = proteolipoprotein; SCI = spinal cord injury; WD = Wallerian degeneration

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Introduction

Degeneration of an axon distal to a point of injury was first described by Waller in degenerating frog peripheral nerves and subsequently termed Wallerian degeneration (WD) (Waller, 1850). In the lesioned PNS, WD may be followed by successful axonal regeneration and functional recovery. However, in the lesioned mammalian CNS, there is an initial phase of transient, abortive sprouting, but no significant axonal regeneration (Schwab and Bartholdi, 1996). In both PNS and CNS, the early phase of WD includes the granular disintegration of the cytoskeleton, in which the cytoskeletal

proteins of the axon, such as microtubules and neurofilaments, are rapidly degraded to granular and amorphous debris. In the PNS and CNS of experimental animals, this process occurs between 18 and 48 h after injury and progresses in a centrifugal pattern away from the lesion site (Griffin *et al*., 1992; George and Griffin, 1994*b*). These early events are probably triggered by the activation of proteases, such as calpains, via an increase in intra-axonal calcium (Schlaepfer, 1974; Waxman *et al*., 1991; George *et al*., 1995). Furthermore, recent studies suggest an involvement of the ubiquitin–proteasome system during these early events (Coleman and Perry, 2002; Zhai *et al*., 2003).

In the PNS, partially overlapping these events, axonal injury leads to the rapid breakdown and phagocytosis of the nerve fibres and their myelin sheaths by endogenous and invading macrophages as well as by local Schwann cells (Bruck, 1997; Shamash *et al*., 2002). Following injury, Schwann cells in the distal nerve stump de-differentiate, proliferate and align within basal lamina tubes (forming bands of Büngner) where they express surface adhesion molecules and neurotrophic factors that promote and guide axonal regeneration (Stoll and Muller, 1999). In the CNS, circulating macrophages invade the degenerating fibre tracts in much lower numbers and more slowly when compared with the PNS (Aldskogius and Kozlova, 1998; Stoll and Jander, 1999). A more prominent role is played by the endogenous microglia, which become activated to form macrophages that are responsible for the phagocytosis of axonal and myelin debris. The time course of the removal of the degenerated tissue is greatly delayed in the CNS as compared with the PNS (Griffin *et al*., 1992; George and Griffin, 1994*a*). Furthermore, in contrast to Schwann cells in the PNS, oligodendrocytes of the CNS do not proliferate but instead undergo apoptosis during the first weeks (Crowe *et al*., 1997; Shuman *et al*., 1997), such that their numbers are reduced by up to 50% in the white matter tracts undergoing WD (Beattie *et al*., 2002).

Myelin-associated proteins (MAPs), such as NOGO-A, have been shown to play significant roles in preventing axonal regeneration in the CNS, both *in vitro* and *in vivo* (Liu *et al*., 2002; Wang *et al*., 2002; Simonen *et al*., 2003; Schwab, 2004). The prolonged persistence of these proteins in degenerating fibre tracts could be a factor contributing to the different regenerative responses in the lesioned CNS and PNS. Recent experimental studies have revealed that individual components of CNS myelin are degraded at different rates during WD of spinal cord white matter tracts. Proteins located in the peri-axonal myelin membrane [e.g. myelin-associated glycoprotein (MAG)], were degraded much more rapidly than those in compact myelin [e.g. myelin basic protein (MBP) and proteolipoprotein (PLP)] or those in the outer myelin membrane [e.g. myelin oligodendrocyte glycoprotein (MOG)] (Buss and Schwab, 2003). Investigations using post-mortem human spinal cord have revealed the presence of MBP-positive structures within degenerating white matter tracts for a number of years after stroke or traumatic spinal cord injury (SCI) (Buss *et al*., 2004). However, the fate of other myelin-associated molecules, with known inhibitory functions on axon regeneration, remained uncertain. In the present investigation, the spatio-temporal loss of MAG, NOGO-A, PLP and MOG as well as oligodendrocytes has been investigated in samples of post-mortem human spinal cord, taken from patients who died at a range of times following either stroke or traumatic SCI.

Material and methods

Post-mortem

The spinal cords were removed from four control patients who had not suffered from any neurological disease and from 22 patients who died at a range of time points after either cerebral infarction or traumatic SCI. The study was approved by the Aachen University Ethics Committee and subjects' families gave informed consent. Patients with cerebral infarction had a massive infarction in the territory of the middle cerebral artery with severe hemiparesis on the contralateral side (Table 1). Patients with traumatic injury had been diagnosed as having 'complete' injuries and presented with paraplegia or tetraplegia (Table 2). The spinal columns were removed at autopsy, ~15–48 h after death. Following incision of the dura mater, the spinal cord was fixed in 10% buffered formalin for at least 2 weeks. Thereafter, blocks of the lesion site and/or tissue

Table 1 *Patients who died after cerebral infarction by the occlusion of the middle cerebral artery*

Case no.	Age (years)	Side of infarction	Injury–death interval
1	36	Left	3 days
2	63	Right	4 days
3	62	Left	4 days
4	78	Left	7 days
5	76	Left	8 days
6	74	Left	14 days
7	45	Right	5 weeks
8	84	Left	4 months
9	79	Left	3 years

Table 2 *Patients who died after traumatic injury to the spinal cord*

Case no.	Age (years)	Injury level	Injury–death interval
1	21	T12	2 days
2	51	C1	4 days
3	84	C3–4	5 days
4	65	C5	8 days
5	63	C6	11 days
6	18	T6	12 days
7	72	T11–12	24 days
8	85	C3	4 months
9	80	C5–6	1 year
10	44	L1	8 years
11	71	C3–4	20 years
12	47	T5	26 years
13	57	T3–4	30 years

from regions rostral and caudal to the lesion (~1 cm thickness) were embedded in paraffin wax.

Immunohistochemistry

Transverse sections (5 μm thick) were collected onto poly-L-lysine-coated slides and allowed to dry. Sections were de-waxed in xylene and rehydrated. Microwave treatment in 10 mM citrate buffer (pH 6) for 3 × 3 min was followed by blockade of non-specific binding by incubation in 0.1 M phosphate-buffered saline (PBS) containing 3% normal goat serum and 0.5% Triton X-100 for 30 min. Sections were incubated subsequently in the primary antibody (for double immunofluorescence, both primary antibodies were applied together), overnight at room temperature. The antibodies against MAG, NOGO-A and PLP are well characterized and known to give specific staining in human material: mouse anti-MAG (D3A2G5 supernatant; Gabriel *et al*., 1998), mouse anti-NOGO-A (11C7C7 100 μg/ml; Oertle *et al*., 2003; Buss *et al*., 2005) and mouse anti-PLP (Serotec, Oxford, UK, 1 : 500; Lucchinetti *et al*., 1999). The mouse monoclonal antibody against MOG (8-18C5 supernatant; Linnington *et al*., 1984) specifically stains its protein in rat material; furthermore, the epitope recognized by the antibody is completely conserved between rat and human (Breithaupt *et al*., 2003). For axonal and astrocytic staining, a rabbit anti-neurofilament (Sigma-Aldrich 1 : 1000) and a rabbit anti-glial acidic fibrillary protein (GFAP) (DAKO 1 : 1000) antibody were used. Following extensive rinsing steps in 0.1 M PBS, sections processed for single immunofluorescence were incubated in red-fluorescent Alexa 594-conjugated goat anti-mouse antibody (diluted 1 : 500, Molecular Probes) for 1 h at room temperature. For double immunofluorescence, sections were incubated with a combination of Alexa 594-conjugated goat anti-mouse antibody (diluted 1 : 500) and Alexa 488-conjugated goat anti-rabbit antibody (diluted 1 : 500, Molecular Probes). Finally, nuclei were stained for 5 min with 4′,6-diamidino-2-phenylindole (DAPI; diluted 1 : 1000, Sigma) and sections were coverslipped in PBS containing 50% glycerine. For negative controls, the primary antibodies were omitted.

Semi-quantification of oligodendrocytes in long-term pathological cases

Oligodendrocytes were counted in cases with survival times >5 years to ensure complete disappearance of myelin debris and phagocytic macrophages. Cases 10, 12 and 13 (see Table 2) were chosen because they represented a lesion site at the lumbar or thoracic levels, thereby leading to degeneration of the fasciculus gracilis which was also investigated in a corresponding animal study (Beattie *et al*., 2002). The area of the fasciculus gracilis from the four unlesioned cases served as controls. Two sections from every case with a distance of at least 100 μm between them were stained by immunofluorescence for NOGO-A and DAPI (see above). Three sample areas of 374 μm^2 each were chosen from the fasciculus gracilis in every section, and oligodendrocytic cell profiles were counted in these areas (identified by morphology and clear overlap of NOGO-A immunoreactivity with DAPI nuclear staining). The total number of identified cells is presented in Table 3.

Results

Samples of spinal cord from 26 individuals were examined using MAG, NOGO-A, PLP and MOG immunohistochemistry. The brains of all cases were carefully examined. Those obtained from control cases were declared to be without pathological findings. Patients who suffered cerebral infarction had a massive vascular insult in the region of the middle cerebral artery and cases of spinal trauma revealed maceration of the lesion site (not shown). The cases have been subdivided into three groups according to the post-insult survival times (i.e. early, intermediate and late survival times), because distinct morphological stages in the course of WD were found.

Table 3 *Semi-quantification of oligodendrocytes in control and long-term pathological cases*

Cases	No. of oligodendrocytes per 374 μm^2					
	Section 1			Section 2		
Ctrl 1	7	7	9	8	7	8
Ctrl 2	8	5	8	6	8	7
Ctrl 3	10	8	7	9	8	9
Ctrl 4	6	5	7	6	6	6
Case 10	3	5	4	4	3	5
Case 12	4	3	4	3	4	4
Case 13	5	4	4	3	4	4

The number of oligodendrocytes within each field of analysis (374 μm^2) was counted in the fasciculus gracilis of the four control cases (Ctrl 1–4) and of three cases of traumatic SCI with long survival times (cases 10, 12 and 13). Three regions on each of two slides were taken and the mean value of the control and the pathological cases compared: controls 7.29; cases 3.88.

Normal distribution of MAPs in the spinal cord

Staining for MAG (situated in the peri-axonal myelin ring), PLP (situated in compact myelin) and MOG (situated in the outer myelin membrane) revealed evenly distributed myelin rings without immunoreactive cell bodies. The anti-NOGO-A antibody not only demonstrated the inner and outer myelin membranes but also oligodendrocytic cell bodies (Fig. 1). In the grey matter, motor neurons and some interneurons were also NOGO-A positive (not shown).

Early survival times (2–14 days post-insult)

Two days after traumatic injury to the spinal cord, sections from one segment above and below the lesion site displayed a rather heterogeneous appearance, with degenerating and massively swollen axonal profiles in between morphologically normal-appearing nerve fibres. Immunohistochemistry for myelin components demonstrated a similar picture, but with intact myelin sheaths intermingled with swollen and degenerating structures (Fig. 2). Further away from the point of injury, the staining pattern of the four MAPs in the lesioned fibre tracts appeared normal compared with the unaffected regions of white matter and control preparations. This staining pattern of homogeneously distributed myelin rings and NOGO-A-positive oligodendrocytes remained unchanged in the lesioned fibre tracts over the first 12 days after stroke and traumatic SCI.

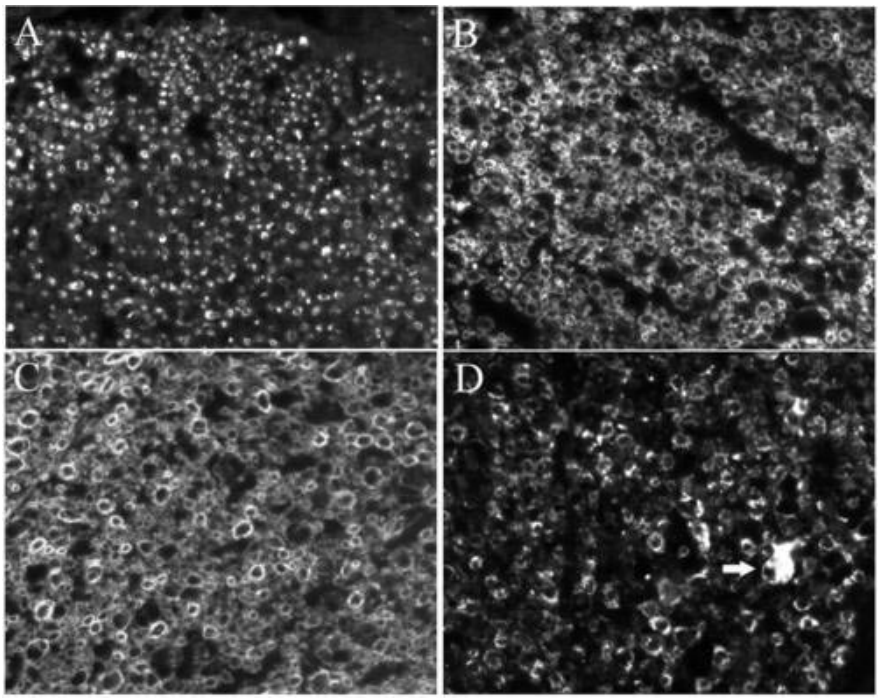

Fig. 1 Myelin-associated proteins in control human spinal cord white matter. Images are transverse sections of the CST of control human spinal cords. In (**A**), immunohistochemistry for MAG, situated on the peri-axonal myelin membrane, demonstrates a homogeneous distribution of myelin rings. (**B**) The appearance of compact myelin is demonstrated with staining for PLP. (**C**) The even distribution of the outer myelin membrane is shown with immunohistochemistry for MOG. (**D**) Apart from its presence on the inner and outer myelin membrane, NOGO-A is also found in oligodendrocytic cell bodies (arrow). The presence of NOGO-A on the inner myelin membrane can only rarely be visualized in human tissue; therefore detectable changes in the distribution of this protein have to be attributed to the outer membrane (**A**–**D**, magnification ×400).

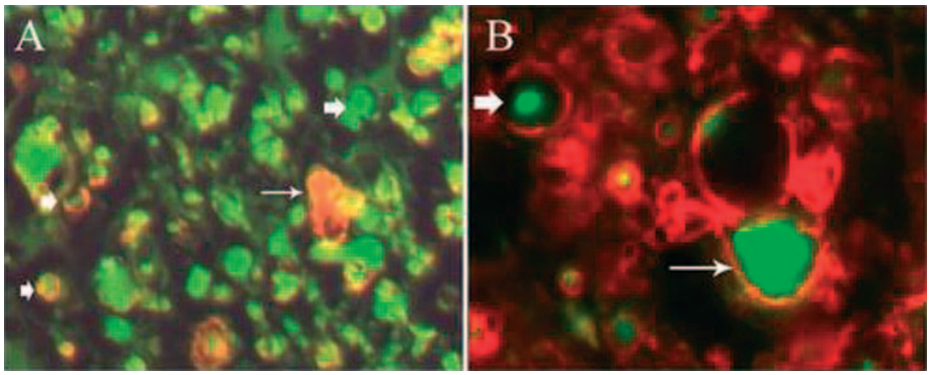

Fig. 2 Axonal and myelin-associated proteins in traumatic SCI and WD. Double immunofluorescence for neurofilament (green) and PLP (red). In **(A)**, the white matter close to the lesion site from a patient who died 2 days after traumatic SCI demonstrates a heterogeneous picture with large, irregular structures (thin arrow) in between morphologically intact axons and myelin sheaths (thick arrow). (**B**) Fourteen days after stroke, in the degenerating CST, large irregular axonal structures surrounded by swollen but morphologically intact myelin rings (thick arrow) are visible within morphologically intact axons with surrounding myelin sheaths (thin arrow) (**A** and **B**, magnification ×560).

The first indication of pathological changes of myelin in degenerating fibre tracts was observed in sections from cervical levels from a patient who died 14 days after stroke. There was a loss of MAG staining on the inner myelin ring of the degenerating corticospinal tract (CST). The staining pattern became heterogeneous, with swollen, irregular profiles in between normal-appearing myelin rings (Fig. 3). The normal pattern of NOGO-A immunohistochemistry, situated on both inner and outer myelin rings (A. Buss *et al*., 2005) could not be clearly identified in the pathological material of the present investigation. Therefore, changes of the inner myelin ring are restricted to alterations in MAG

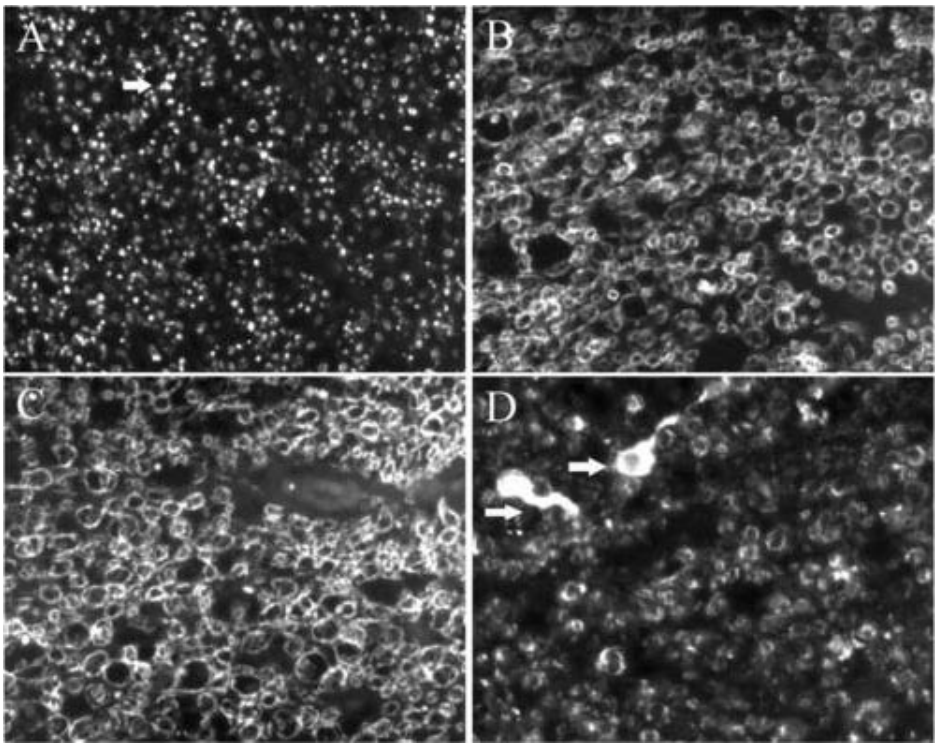

Fig. 3 Myelin-associated proteins in degenerating nerve fibre tracts 14 days after injury**.** Images are transverse sections of the degenerating CST of a patient who died 14 days after stroke. (**A**) Immunohistochemistry for MAG demonstrates a slight reduction in the number of periaxonal myelin rings compared with control cases. Instead some irregular immunopositive structures can be seen (arrow). (**B**) Fourteen days after cerebral infarction, the homogeneous distribution of PLP-positive rings is unchanged compared with control cases. (**C**) Fourteen days after stroke, no changes in MOG immunoreactivity can be detected. (**D**) NOGO-A staining demonstrates the still homogeneous distribution of the outer myelin rings and oligodendroglial cell bodies (arrows) (**A**–**D**, magnification ×400).

staining. The immunoreactivity for PLP and MOG as well as for NOGO-A-positive oligodendroglial cell bodies remained evenly distributed (Fig. 3). However, some myelin rings appeared swollen and they surrounded enlarged, irregular neurofilament-positive structures, most probably reflecting end bulbs from degenerating axons (Fig. 2).

Intermediate survival time (24 days to 3 years post-insult)

In these cases, pathological changes in degenerating fibre tracts were visible over the whole length of the spinal cord and it was difficult to distinguish between sections obtained from regions close to or remote from the point of injury. Sections from a patient who died 24 days after Th11/12 traumatic injury showed clear signs of degeneration for all four MAPs investigated in the ascending dorsal column up to cervical levels. The density of myelin rings was reduced, and numerous amorphous structures, which most probably reflected the presence of myelin debris, were visible. At 5 weeks after stroke, the pathological changes were very similar in the descending affected CST (Fig. 4).

Four months after traumatic SCI and stroke, the amount of morphologically intact ring-like myelin structures was reduced further in both ascending and descending fibre tracts undergoing WD, whilst highly immunoreactive debris was clearly visible (not shown). In cases with survival times of 10 months and longer after injury, the degenerating tracts were almost devoid of MAG immunoreactivity (Fig. 5). However, even in the degenerating CST of patients with survival times of up to 3 years after injury, immunohistochemistry for PLP, MOG and NOGO-A still demonstrated occasional immunopositive myelin rings in between amorphous structures. Furthermore, NOGO-A staining demonstrated the continued presence of oligodendrocytic cell bodies in the affected fibre tracts (Fig. 5). Double staining for myelin proteins and neurofilament demonstrated that the few remaining ring-like structures were no longer associated with neurofilament-positive axons (Fig.6).

Long survival time (8 years to 30 years post-insult)

In these cases, all myelin proteins investigated were almost completely absent from degenerated white matter tracts rostral or caudal to the lesion. The myelin debris had been cleared and ring-like structures were no longer visible (data not shown). However, immunohistochemistry for NOGO-A still demonstrated oligodendrocytic cell bodies within the degenerated ascending and descending fibre tracts. In two cases, with survival times of 26 and 30 years after thoracic SCI, the fasciculis gracilis appeared completely degenerated

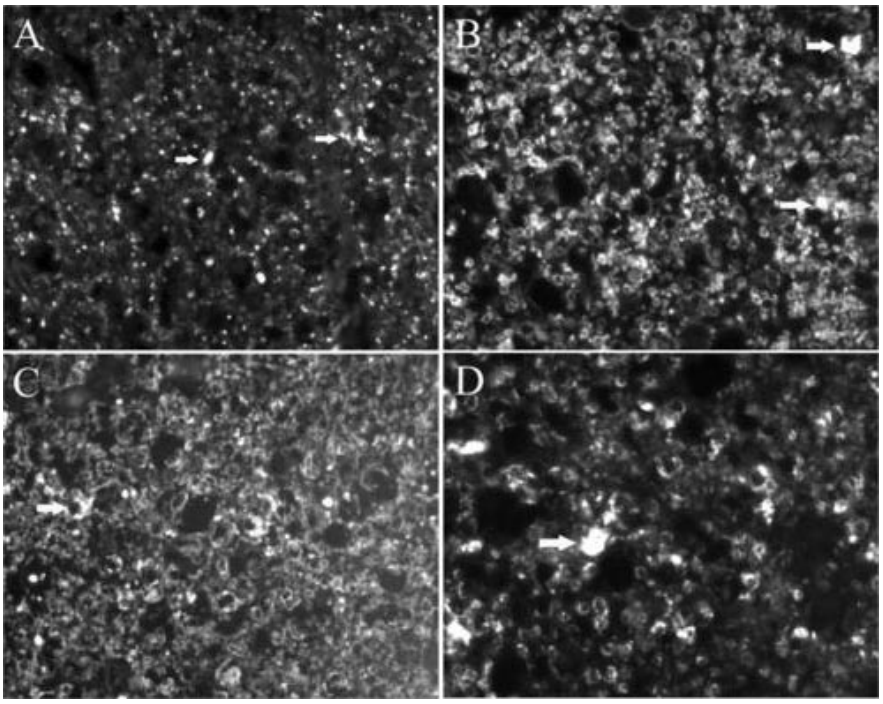

Fig. 4 Myelin-associated proteins in degenerating nerve fibre tracts 5 weeks after injury. Images are transverse sections of the degenerating CST of a patient who died 5 weeks after stroke. (**A**) Immunohistochemistry for MAG demonstrates a dramatic reduction in the number of peri-axonal myelin rings, and only a few irregular strongly immunopositive structures can be seen (arrows). (**B**) Five weeks after cerebral infarction, PLP staining demonstrates a clear reduction in the amount of compact myelin rings, many with an irregular appearance. In between, highly immunoreactive irregular profiles can be seen most probably representing degenerated myelin debris (arrows). (**C**) The number of MOG-positive outer myelin rings is also reduced; instead myelin debris is visible (arrow). (**D**) NOGO-A immunohistochemistry demonstrates oligodendrocytes (arrow) in between a heterogeneous picture of myelin debris and still morphologically normal-appearing myelin rings (**A**–**D**, magnification ×400).

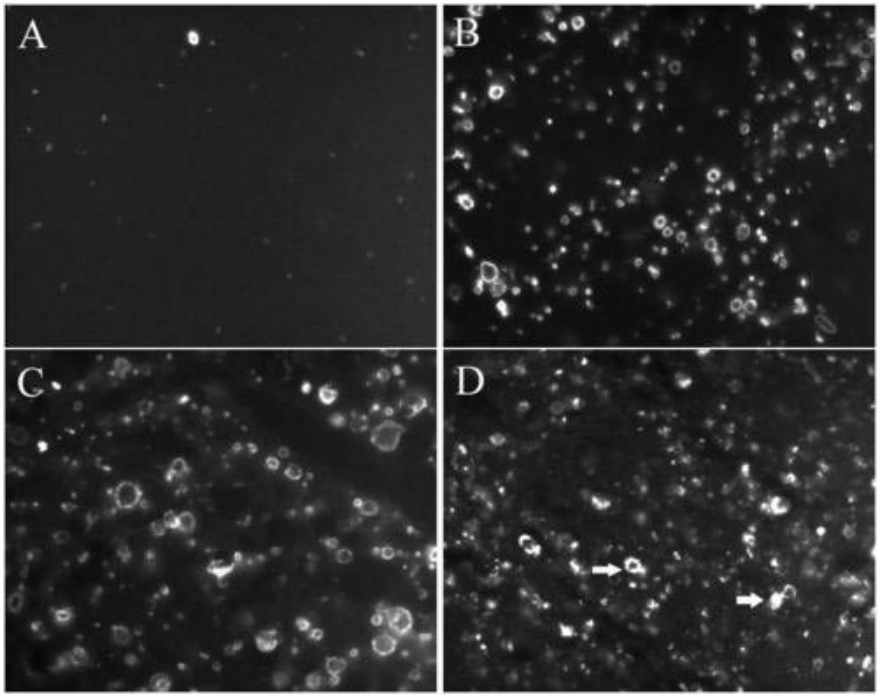

Fig. 5 Myelin-associated proteins in degenerating nerve fibre tracts 3 years after injury. Images are transverse sections of the degenerating CST of a patient who died 3 years after stroke. (**A**) The degenerating fibre tract is devoid of MAG-immunopositive structures. (**B** and **C**) PLP and MOG staining demonstrates ring-like myelin structures in the degenerating CST at this time point. (**D**) NOGO-A immunohistochemistry demonstrates myelin rings and some oligodendrocytic cell bodies (arrows) (**A**–**D**, magnification ×400).

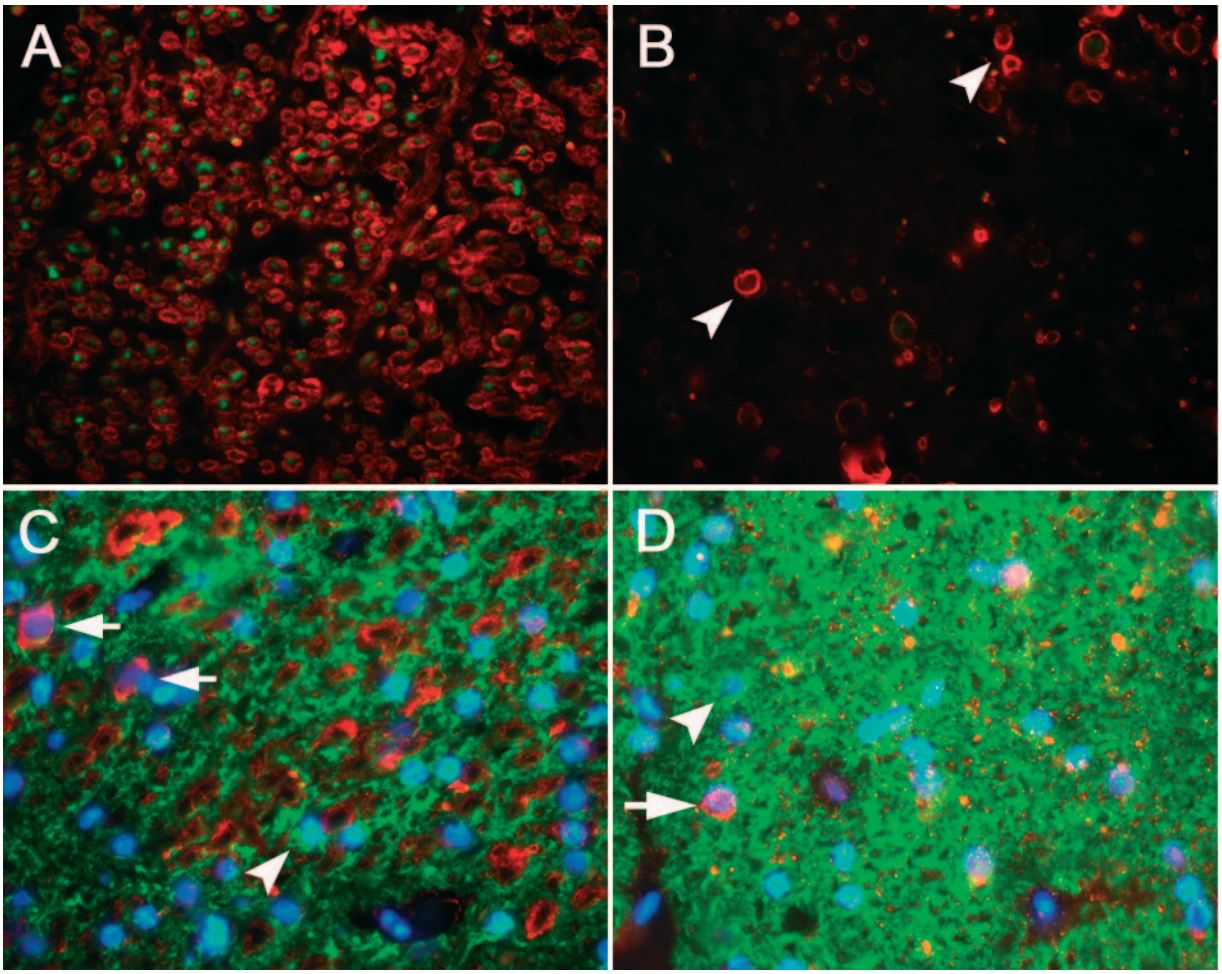

Fig. 6 Oligodendrocytes and astrocytes in unaffected and degenerated white matter tracts. (**A** and **B**) Double immunofluorescence with PLP (red) and neurofilament (green) on transverse sections from a patient who died 3 years after stroke. (**A**) In the unaffected CST, a homogeneous picture can be seen of axonal profiles surrounded by myelin rings. (**B**) In the degenerated CST, no neurofilament-positive axonal structures are visible; only a few remaining ring-like myelin structures can be seen (arrowheads). (**C** and **D**) Triple immunofluorescence with NOGO-A (red), GFAP (green) and DAPI (blue) on sections from a patient who died 26 years after traumatic SCI at Th5. (**C**) The unaffected cuneate tract shows oligodendrocytes (arrows) and their myelin sheaths in between the network of astrocytes (arrowhead) and their processes. (**D**) The degenerated gracile tract demonstrates rare oligodendroglial cell bodies (arrow) without ring-like structures in between the dense irregular GFAP-positive astrocytic scars; astroglial cell bodies are hardly detectable (arrowhead) (**A**–**D**, magnification ×400).

whereas the fasciculus cuneatus remained intact. Oligodendrocyte cell bodies were counted in the cervical gracile tracts of the four control cases and three pathological cases with a lumbar or thoracic lesion site at survival times between 8 and 30 years (see Table 3). The results indicate a 40–50% reduction of the oligodendrocytes in the degenerated dorsal columns. Double immunofluorescence for NOGO-A and GFAP within such long-term degenerated white matter tracts revealed that surviving oligodendrocytes were surrounded by densely packed astrocytic scar tissue (Fig. 6).

Discussion

In the present study, the temporal changes of a number of maps were investigated during WD of white matter fibre tracts in the human spinal cord. The data demonstrate that the sequence of loss of myelin proteins during WD is similar to that described in experimental animals but that it follows a greatly extended time course. Oligodendrocytes can still be detected in degenerated fibre tracts long after injury; however, their number is reduced by 40–50%.

Spinal cord immediately adjacent to the primary lesion site

In spinal cord tissue adjacent to the acute lesion, the consequences of direct injury and tissue destruction became gradually less prominent with increasing distance from the injury site. At 1 day after injury, changes in the staining pattern of axonal proteins and MAPs were found: irregular structures, most probably reflecting end bulbs and axonal beading, as well as swollen and disorganized myelin could be observed

amongst normal-looking fibres. The early changes around the lesion site may be attributed to primary damage characterized by breakdown of the blood–brain barrier, inflammation and macrophage invasion and especially to secondary injury mechanisms such as ischaemia and excitotoxicity (Dusart and Schwab, 1994; Amar and Levy, 1999; Mautes *et al*., 2000). At segmental levels more remote from the injury site, the pathological changes reflected the pattern of specific events that were associated with the spread of WD.

Fibre tracts undergoing WD after SCI or stroke

The time course of myelin changes in WD is often investigated using either electron microscopy or general myelin stains such as luxol fast blue. In experimental animals, morphological changes first become visible in the degenerating tracts ~3 days after injury and consist of more loosely wrapped myelin lamellae, eventually leading to segmentation of the myelin sheath into ovoids (George and Griffin, 1994*b*; Wroblewski *et al*., 2000). These ovoids are then slowly removed, leading to complete resolution within a few months (Stoll *et al*., 1989; Stoll and Jander, 1999). Recently, immunohistochemical analysis following experimental spinal cord lesions has revealed a differential pattern of myelin protein loss in dorsal columns undergoing WD. Axonal cytoskeletal proteins and proteins located on the peri-axonal myelin membrane were lost within the the first 2 weeks after injury. However, the loss of proteins from compact myelin and the outer myelin membrane was delayed. Even 2 months after injury, myelin structures were clearly detectable in the degenerating fibre tracts (Buss and Schwab, 2003). In human CNS nerve fibre tracts undergoing WD, luxol fast blue or Marchi staining has demonstrated the loss of myelin over a period of ~2 years (Miklossy and Van der Loos, 1991; Becerra *et al*., 1995). Furthermore, immunohistochemical analyses in post-mortem tissue has revealed the presence of MBP for up to 3 years in degenerating spinal cord fibre tracts (Buss *et al*., 2004). The present investigation extends previous reports by demonstrating the sequential and extended loss of MAG, PLP, MOG and NOGO-A.

Between 14 days and 4 months after insult, MAG immunoreactivity was gradually lost from the inner myelin lamella. The concomitant loss of axonal neurofilament underlines the close inter-relationship between the axon and its directly apposing myelin membrane, and suggests a uniform mechanism of destruction. The initial phase of axonal degeneration is due to the activation of endogenous proteases, such as calpains (George *et al*., 1995; Coleman and Perry, 2002). We propose that the same mechanism is involved in the destruction of the peri-axonal myelin membrane. It is possible that activated calpains, having leaked from the degenerating axon, may have attacked the inner myelin membrane. Previous investigations have demonstrated that activated microglia and macrophages are absent from the degenerating tracts during the first weeks after injury (Schmitt *et al*., 1998), and it is unlikely that they contributed to this early and specific pattern of myelin degeneration.

Compact myelin and the outermost myelin membrane appeared largely unaffected during the early time points after insult. The first indications of compact and outer myelin degeneration were detected at 5 weeks after injury. This corresponds spatially and temporally with the appearance of CD68-positive macrophages within these degenerating fibre tracts (Schmitt *et al*., 1998) and reflects the probable involvement of these cells in later degenerative events. The progressive loss of myelin structures continued over the first 2 years, with debris and occasional myelin rings still being detectable 3 years after injury.

Oligodendrocytes, as the myelin-producing cells in the CNS, undergo apoptotic cell death during the first weeks of WD. This process has been detected in both ascending and descending fibre tracts in a number of species including rats, monkeys and humans, and has been reported to proceed from day 1 after injury up to survival times of 8 weeks (Crowe *et al*., 1997; Shuman *et al*., 1997; Emery *et al*., 1998). Recently, a study in mice demonstrated a 50% reduction in the number of oligodendrocytes at 'long survival times' (up to 42 days) during WD (Beattie *et al*., 2002). The present investigation in post-mortem human material also demonstrated a reduction of NOGO-A-positive oligodendrocytes in long-term degenerated white matter tracts. The semi-quantitative analysis of selected cases with long survival times revealed a 40–50% reduction in the number of oligodendroglial cells in degenerated dorsal columns when compared with control cases. In our material, we did not adapt our data to the eventual atrophy taking place in fibre tracts undergoing WD. Thus, the loss of oligodendrocytes could be even higher than the 40–50% reported here. It was not possible to assess the complete time course of oligodendrocyte loss using all of the samples available in the investigation because it proved difficult to identify oligodendroglia unequivocally in the degenerating white matter tracts during the first months after trauma. This was largely due to the presence of myelin (NOGO-A) in phagocytosing macrophages. The present data revealed a population of surviving NOGO-A-positive oligodendrocytes that were deeply embedded within a dense astrocytic scar. This observation supports previous data from our group which reported intense anisomorphic astroglial scarring in human white matter tracts undergoing WD (Buss *et al*., 2004).

In conclusion, the present data reveal a sequential loss of MAPs and loss of oligodendrocytes within human nerve fibre tracts undergoing WD. The pattern of these events is similar to experimental investigations but follows a greatly extended time course, probably due to simple differences in scale between the human spinal cord and that of routinely used experimental animals. These differences between human and experimental tissues are not trivial. The myelin-associated molecules MAG and NOGO-A have both been demonstrated to be potent inhibitors of axon regeneration (Mukhopadhyay *et al*., 1994; Schwab, 2004). Their apparently slow degradation and removal from the injured

spinal cord may contribute to the maintenance of an environment that is hostile to axon regeneration. It is important that investigators developing future intervention strategies are aware of the time course of changes in the properties of the reactive tissues, not only at the lesion site, but also in remote areas.

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